

Appendix III

P03170US/WARF-0204

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23°C during daylight hours (15h) and dropped to 15°C at night. Foliage blight scores were recorded 69, 92, 116, and 163 hours after inoculation. A blight scale, with 0 indicating a dead plant and 9 no visible infection, was used to visually rate disease severity. All the plants were tested in three repetitions. The ratings and the ranges of percentage infections associated with the rating value were as follows: 9, no visible infection; 8, less than 10% infection; 7, 11-25% infection; 6, 26-40% infection; 5, 41 to 60% infection; 4, 61-70% infection; 3, 71-80% infection; 2, 81-90% infection; 1, greater than 90% infection; 0, all dead. Plants with scores of 8 or above were scored as resistant and plants with scores of 6.9 or below were scored as susceptible. Plants with scores between 6.9 and 8 were scored as intermediate resistant.

Transgenic plants with gene 2 were tested using the above method. 69, 92, 116, and 163 hours after inoculation, the average resistant score for transgenic plants with gene 2 was 7.3.

Example 5:

The following example shows an amino acid comparison between the gene 2 coding region from a disease resistant and disease susceptible variety. The top sequence is the gene 2 coding region from the susceptible 177013 homolog. The bottom sequence is the gene 2 coding region from the resistant homolog. Note that on the line numbered 451, at residue 454, a single nucleotide difference between R and S changes a Tyrosine (Y; resistant) to an amber stop codon.

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20      1 MAEAFIQVLLDNLTSLKGLLALLFGFQDEFQRLSSMFSTIQAVLEDAQE 50
      1 MAEAFIQVLLDNLTSLKGLVLLFGFQDEFQRLSSMFSTIQAVLEDAQE 50
25      51 KQLNNKPLENWLQKLNAAATYEVDDILDEYKTRATRESQSEYGRYHPKVIP 100
      51 KQLNNKPLENWLQKLNAAATYEVDDILDEYKTRATRESQSEYGRYHPKVIP 100
30      101 FRHKVGKRMDDQVMKKLKAIAEERKNFHLHEKIVERQAVRRETGSLTEPQ 150
      101 FRHKVGKRMDDQVMKKLKAIAEERKNFHLHEKIVERQAVRRETGSLTEPQ 150
35      151 VYGRDKEKDEIVKILINNVSDAQHLSVLPILGMGGLGKTTLAQMVENDQR 200
      151 VYGRDKEKDEIVKILINNVSDAQHLSVLPILGMGGLGKTTLAQMVENDQR 200
40      201 VTEHFHSKIWICVSEDFDEKRLIKAIVESIEGRPLLGEMLAPLQKKLQE 250
      201 VTEHFHSKIWICVSEDFDEKRLIKAIVESIEGRPLLGEMLAPLQKKLQE 250
45      251 LLNGKRYLLVLDVWNEDQOKWANLRAVLKVGASGASVLTTRLEKVGSI 300
      251 LLNGKRYLLVLDVWNEDQOKWANLRAVLKVGASGASVLTTRLEKVGSI 300
      301 MGTLPQYELSNLSQEDCWLLFMQRAFGHQEEINPNLVAIGKEIVKSGGV 350
      301 MGTLPQYELSNLSQEDCWLLFMQRAFGHQEEINPNLVAIGKEIVKSGGV 350
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351 PLAAKTLGGILCFKREERAWEHVRDSPIWNLFDQESSILPALRLSYHQLP 400
|||||
351 PLAAKTLGGILCFKREERAWEHVRDSPIWNLFDQESSILPALRLSYHQLP 400
5
401 LQKQCFAYCAVFFPKDAKMEKEKLISLWMAHGFLLSKGNMELEDVGDEVW 450
|||||
401 LQKQCFAYCAVFFPKDAKMEKEKLISLWMAHGFLLSKGNMELEDVGDEVW 450
10
451 KEL*LRFFQIEIVKDGKTYFKMHDLIHDLATSLFSANTSSSNIREINKH 500
|||||
451 KEL*LRFFQIEIVKDGKTYFKMHDLIHDLATSLFSANTSSSNIREINKH 500
15
501 SYTHMMSIGFAEVVFFYTLPPLEKEFISLRVNLGDSTFNKLPSSIGDLVH 550
|||||
501 SYTHMMSIGFAEVVFFYTLPPLEKEFISLRVNLGDSTFNKLPSSIGDLVH 550
20
551 LRYLNLYGSGMRSLEPKQLCKLQNLQTLQYCTKLCCLPKETS KLGS LRN 600
|||||
551 LRYLNLYGSGMRSLEPKQLCKLQNLQTLQYCTKLCCLPKETS KLGS LRN 600
25
601 LLLDGSQSLETCMPFRIGSLTCLKT LGQFVVGRRKKGYQLGELGNLNYGSI 650
|||||
601 LLLDGSQSLETCMPFRIGSLTCLKT LGQFVVGRRKKGYQLGELGNLNYGSI 650
30
651 KISHLERVKNDKDAKEANLSAKGNLHSLMSWNNFGPHIYEESEVKVLEA 700
|||||
651 KISHLERVKNDKDAKEANLSAKGNLHSLMSWNNFGPHIYEESEVKVLEA 700
35
701 LKPHSNLTSIKIYGFGRGIHLPEWMNHSLVKNIVSILISNFRNCSCLPFPG 750
|||||
701 LKPHSNLTSIKIYGFGRGIHLPEWMNHSLVKNIVSILISNFRNCSCLPFPG 750
40
751 DLPCLSLSLHWSADVEYVEEVDIDVHSGFFTRIRFP SLRKLDIWDFGS 800
|||||
751 DLPCLSLSLHWSADVEYVEEVDIDVHSGFFTRIRFP SLRKLDIWDFGS 800
45
801 LKGLLKEGEEQFFVLEEMIHECPFLTSSNLRAITSLRICYNKVATSF 850
|||||
801 LKGLLKEGEEQFFVLEEMIHECPFLTSSNLRAITSLRICYNKVATSF 850
50
851 PEEMFKNLANLKYLTI SRNNLKE LPTSLASLNALKSL.....ALES LP 894
|||||
851 PEEMFKNLANLKYLTI SRNNLKE LPTSLASLNALKSLKIQLCCALESLP 900
55
895 EEGLEGLSSLTTEL FVERCNMLKCLPEGLQH LTTLTSLKIRGCPQLIKRCE 944
|||||
901 EEGLEGLSSLTTEL FVERCNMLKCLPEGLQH LTTLTSLKIRGCPQLIKRCE 950
945 KGIGEDWHKISHIPNVNIYI* 965
|||||
951 KGIGEDWHKISHIPNVNIYI* 971

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55 Example 6:

The following example shows a nucleic acid comparison between the gene 2 coding regions from a disease resistant and disease susceptible variety. The top sequence is the gene 2 coding region from the resistant homolog. The bottom sequence is the gene 2 coding region